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13. ABSTRACT ***(Maximum 200 Words)***

This work elucidates the mechanism by which serotonin regulates the circadian clock's response to the phase-adjusting effects of exposure to light. The primary goals are (1) to identify and characterize the neural receptors that mediate these phase-shifting responses, and (2) to determine their sites of action. Using the Syrian hamster, we cloned and characterized the 5-HT1A and 5-HT7 receptors. We then demonstrated that these receptors are present in the suprachiasmatic nucleus (SCN). We sought to determine serotonin receptor mRNA in identified cells within the SCN and retina, but neither of two approaches was successful during the funding period. We studied the effects of various serotonin receptor agonists and antagonists. Results indicate that presynaptic 5-HT1B receptors on retinal terminals in the SCN attenuate the effect of light on melatonin production in the pineal gland, as well as the effect of light on circadian phase. We published three studies on the role of adenosine in the regulation of light-induced phase shifts, and found that blocking A1AR in the SCN promotes wakefulness. Our other studies have begun to elucidate the gene control of signaling cascades in the SCN, and show that light exposure activates distinct cascades in distinct cell groups.

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FINAL REPORT

ASOFR F49620-00-1-0058 (Dec 15, 1999 – Dec 14, 2002)

AFOSR Program Manager: Willard Larkin, PhD

Principal Investigator: Michael A. Rea, PhD

Institution: Circadian Neurobiology Laboratory
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2. Objectives:

a. To clone and characterize the hamster 5-HT1A and 5-HT7 receptors

b. To determine whether 5-HT1A and 5-HT7 receptors are expressed in the hamster SCN and retina.

c. To determine the mechanism by which 5-HT1A/7 receptor agonists modulate RHT neurotransmission in the hamster SCN.

d. To determine the relative contribution of 5-HT1A and 5-HT7 receptors in the modulation of photic phase shifts in the hamster.

e. To determine the mechanism by which 5-HT1A receptor agonists augment photic phase shifts.

3. Status of the Effort: Completed

A certain amount of risk is associated with any move. In my case, however, two major setbacks that occurred subsequent to my relocation from AFRL to the University of Houston substantially impacted research productivity under AFOSR support. First, I experienced considerable delay in the renovation and construction of my laboratory space. Work on my lab space was not completed until November of 2000, thirteen months after arrival of my lab equipment and furniture at the University of Houston. During this period, my lab operated out of cardboard boxes, major equipment items remained in storage, and the lack of availability of an appropriately controlled environment for rodent circadian studies prevented the accomplishment of several first year research goals. We were pleased to occupy the newly renovated laboratory space in December of 2000. Then, on June 9, 2001, flooding in our basement animal facility during Tropical Storm Allison destroyed 1/3 of my research capacity and completely halted our behavioral pharmacology research effort until the facility was finally restored

some 8 months later. These two setbacks combined to either slow or curtail research activity during 21 months of the 36 month funding period.

4. Accomplishments / New Findings:

a. To clone and characterize the hamster 5-HT1A and 5-HT7 receptors

The Syrian hamster 5-HT1A and 5-HT7C receptor genes were cloned and sequenced. Complete coding regions are presented in Table 1. Both receptors were cloned by polymerase chain reaction (PCR) using degenerate primers targeting the amino acid sequences of the rat, mouse, and human receptors. The hamster 5-HT1A receptor is 1269 nucleotides in length and encodes a 442 amino acid polypeptide that is 95% sequence identical to the rat receptor. The hamster 5-HT7C receptor is 1413 nucleotides in length and encodes a 471 amino acid polypeptide that is 96% identical to the rat receptor. In both cases, the majority of the amino acid substitutions relative to the rat sequences occurred in the N-terminal extracellular domain and the third intracellular loop (Figures 1 & 2), neither of which are believed to participate in ligand recognition or binding. Therefore, it seems unlikely that the differences observed between the hamster and rat amino acid sequences of these receptors would result in pharmacologically significant differences in ligand binding preference. However, this conclusion remains to be confirmed by quantitative receptor binding studies. These experiments are currently in progress under support from another agency.

b. To determine whether 5-HT1A and 5-HT7 receptors are expressed in the hamster SCN and retina.

The presence of 5-HT1A, 5-HT1B, 5-HT2C, and 5-HT7 receptor mRNA in the hamster SCN was demonstrated by reverse transcriptase (RT)-PCR in micropunches of hamster SCN collected at 4 different circadian phases (ZT 3, 9, 15, 21). All four transcripts were detected in the hamster SCN (Figure 3a). The abundance of 5-HT7 receptor mRNA showed diurnal variation (Figure 3b) with peak levels occurring during the dark phase (ZT15 & 21). In situ hybridization for 5HT7 mRNA confirmed localization of expression within the hamster SCN (Figure 4). Finally, specific, high-affinity [3H]-8-OH-DPAT binding was detected in the hamster SCN, and approximately 50% of the binding was blocked by either pindolol or ritanserin, indicating the presence of 5HT1A and 5HT7 receptors, respectively (Figure 5).

At this point, we sought to develop a method for detecting serotonin receptor mRNA in identified cells within the SCN and retina. Two approaches were pursued; (1) single cell RT-PCR using mRNA extracted from retinorecipient SCN neurons identified in the hypothalamic slice preparation, and (2) isolation of identified neuronal populations within the SCN by laser capture microdissection. Neither approach was successful during the funding period.

c. To determine the mechanism by which 5-HT1A/7 receptor agonists modulate RHT neurotransmission in the hamster SCN.

The effects of a variety of serotonin receptor agonists and antagonists on excitatory postsynaptic currents evoked in retinorecipient SCN neurons in the hypothalamic slice preparation were determined. Serotonin (5-HT), 5-carboxyamidotryptamine (5-CT), and (+/-) 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) all dose dependently attenuated EPSC amplitude (e.g., Figure 6). The inhibitory effect of 8-OH-DPAT was blocked by the selective serotonin receptor antagonists, methiothepin, clozapine and DR4004 (Figure 7), but not by WAY 100,635 (not shown). This pattern of antagonism is consistent with the involvement of a 5-HT7 receptor. Finally, concentrations of 8-OH-DPAT that elicited 80% inhibition of evoked EPSCs failed to attenuate the response to glutamate (0.2 micromolar) applied by pressure ejection from a micropipette placed in the vicinity of the patched neuron (Figure 8). This observation suggests that presynaptic receptors are responsible, in part, for the response to 8-OH-DPAT. A manuscript describing these results is in preparation.

In order to directly investigate presynaptic effects of serotonin receptor agonists, we attempted to develop an optical recording strategy in which presynaptic terminals are labeled with a calcium sensitive dye and recorded simultaneously with EPSCs during whole cell patch recording experiments. Unfortunately, we have not yet succeeded with this approach.

d. To determine the relative contribution of 5-HT1A and 5-HT7 receptors in the modulation of photic phase shifts in the hamster.

Beyond the pharmacological and molecular studies described above, we have not pursued this aim.

e. To determine the mechanism by which 5-HT1A receptor agonists augment photic phase shifts.

Delays and disasters (described above) conspired to limit opportunities to pursue this aim. Considerable time was spent preparing MRN lesioned animals to test the site of action of the serotonergic drug BMY7378, which we reported to greatly augment light induced phase advances in hamsters. Unfortunately, a large study involving 48 carefully lesioned animals was lost during the flood of 2001. After the flood, Dr Weber left the group to accept a position at Rider College. We have not followed up on this work.

f. Studies not included in the original specific aims

Effects of 5-HT1B receptor agonists on photic suppression of melatonin synthesis: Serotonin (5-HT) modulates the phase adjusting effects of light on the mammalian circadian clock, in part, through the activation of presynaptic $5HT_{1B}$ receptors located on retinal terminals in the suprachiasmatic nucleus (SCN). A study was conducted to determine whether activation of $5\text{-}HT_{1B}$ receptors also alters photic regulation of

nocturnal pineal melatonin production (Rea & Pickard, 2000; PDF included). Systemic administration of the $5HT_{1B}$ receptor agonist TFMPP attenuated the inhibitory effect of light on pineal melatonin synthesis in a dose-related manner with an apparent ED_{50} value of 0.9 mg/kg. The effect of TFMPP on light-induced melatonin suppression was blocked by the $5HT_1$ receptor antagonist, methiothepin, but not by the $5HT_{1A}$ antagonist, WAY 100,635, consistent with the involvement of $5HT_{1B}$ receptors. The results are consistent with the interpretation that activation of presynaptic 5-HT_{1B} receptors on retinal terminals in the SCN attenuates the effect of light on pineal melatonin production, as well as on circadian phase.

5-HT1B receptor knockout animals: A project initiated prior to funding and completed during the current funding period involved an investigation of the effects of constant light on circadian period in 5-HT1B knockout mice. This study revealed that the increase in free running period under constant illumination that is typically observed in wild type mice is absent in 5-HT1B knockout mice. This finding suggests that the period lengthening effect of light is mediated through the serotonergic system and mediated in part by 5-HT1B receptors. The study was published in the Journal of Biological Rhythms (Sollars et al., 2001; PDF included).

Neurochemistry of Photic Entrainment: Although we have vigorously pursued the work proposed under the original specific aims of F49620-00-1-0058, recent developments in the field, and interesting new data from our own laboratory, have precipitated a re-evaluation of our working model for regulation of RHT neurotransmission in the Syrian hamster. We have developed a new, three compartment model to guide experimentation during the current and future research efforts. We also expanded our investigation of the interaction between serotonin and glutamate in the suprachiasmatic nucleus (SCN) to include nitric oxide (NO) production, which we have previously shown to be required for both glutamate- and light-induced phase shifts in hamsters and rats. We propose that glutamate release, which is dynamically regulated by serotonin and adenosine A1 receptors located on retinohypothalamic tract (RHT) terminals, mediates light-induced phase alterations of the SCN circadian clock through the activation of nitric oxide synthase (NOS). We propose that this process occurs in a non-oscillatory population of "entrainment cells", which express the immediate early gene FOS in response to brief nocturnal light exposure. We further propose that these phase altering signals are communicated from entrainment cells to clock cells by nitric oxide, in part through direct activation of the PKG and MAPK pathways.

Three studies designed to evaluate the role of adenosine in the regulation of light induced phase shifts and RHT neurotransmission were completed and published. The first study (Elliott et al., 2001) investigated the hypothesis that adenosine A_1 receptors modulate the phase adjusting effect of light on the circadian clock. Systemic administration of the selective adenosine A_1 receptor agonist, N^6-cyclohexyl-adenosine (CHA), significantly ($p<0.05$) attenuated light-induced phase delays and advances of the circadian activity rhythm. Selective agonists for the adenosine A_{2A} and adenosine A_3 receptors were without effect. The inhibitory effect of CHA on light-induced phase advances was dose-dependent (0.025 – 1.0 mg/kg, ED_{50} = 0.3 mg/kg), and this effect was

blocked in a dose-dependent (0.005 – 1.0 mg/kg) manner by the adenosine A_1 receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX). Injection of CHA (10 μM) into the region of the suprachiasmatic nucleus significantly attenuated light-induced phase advances, and this effect was also blocked by DPCPX (100 μM). The results suggest that adenosine A_1 receptors located in the region of the suprachiasmatic nucleus regulate the response of the circadian clock to the phase-adjusting effects of light.

In a related study, we collected pharmacological evidence supporting the involvement of adenosine A1 receptors in the regulation of the response of the circadian clock to light in mice (Sigworth & Rea, 2003). Systemic injection of the selective adenosine A1 receptor agonist, N^6-cyclohexyladenosine (CHA; 0.3 mg/kg) resulted in a 49% reduction ($p<0.05$) in the magnitude of light-induced phase delays. The inhibitory effect of CHA on light-induced phase delays was dose dependent over a range of 0.1 to 5 mg/kg with an apparent EC_{50} of 0.3 mg/kg. Prior administration of the selective adenosine A1 receptor antagonist dipropylcyclopentylxanthine (DPCPX; 1 mg/kg) completely blocked the effect of CHA on photic phase delays. Finally, CHA significantly attenuated light-induced phospho-ERK immunoreactivity in the mouse SCN, consistent with a mode of action involving events that occur early in the signaling cascade through which photic information is conveyed to the circadian clock. These data indicate that the role of adenosine in the regulation of circadian phase is similar in mice and hamsters.

The third study investigated the mechanism by which A1AR agonists inhibit light-induced phase shifts by concentrating on the effects of the drugs at the RHT synapse (Hallworth et al., 2002). Intracellular recordings were made from SCN neurons in slices of hamster hypothalamus using the *in situ* whole-cell patch clamp method. A monosynaptic, glutamatergic, excitatory postsynaptic current (EPSC) was evoked by stimulation of the optic nerve. The EPSC was blocked by bath application of the adenosine A_1 receptor agonist cyclohexyladenosine (CHA) in a dose-dependent manner with a half-maximal concentration of 1.7μM. The block of EPSC amplitude by CHA was antagonized by concurrent application of the adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX). The adenosine A_{2A} receptor agonist CGS21680 was ineffective in attenuating the EPSC at concentrations up to 50μM. Trains of four consecutive stimuli at 25ms intervals usually depressed the EPSC amplitude. However, after application of CHA, consecutive responses displayed facilitation of EPSC amplitude. The induction of facilitation by CHA suggested a pre-synaptic mechanism of action. After application of CHA, the frequency of spontaneous EPSCs declined substantially, while their amplitude distribution was unchanged or slightly reduced, again suggesting a mainly pre-synaptic site of action for CHA. Application of glutamate by brief pressure ejection evoked a long-lasting inward current which was unaffected by CHA at concentrations sufficient to reduce the evoked EPSC amplitude substantially (1 to 5μM), suggesting that post-synaptic glutamate receptor-gated currents were unaffected by the drug. Taken together, these observations indicate that CHA inhibits optic nerve-evoked EPSCs in SCN neurons by a predominantly pre-synaptic mechanism.

Microdialysis experiments: We have developed an HPLC method for detecting picomolar quantities of serotonin, adenosine, and the oxidative products of NO, NO_2 and NO_3 (NOx), in a single,10 microliter microdialysis sample. All four analytes are detected during a 7 minute run, enabling quantitative measurement of these substances in near real time. Glutamate levels in the same dialysates are determined in a separate analysis. We have shown that (1) exogenous glutamate or NMDA administered through the dialysis probe increases NOx production in the hamster SCN, (2) brief light exposure during the early subjective night also appears to increase NOx production, and (3) the effects of both the excitatory amino acids, and of brief light exposure, are blocked by NOS inhibitors. Furthermore, we have confirmed our preliminary observation that (4) infusion of DPCPX, a highly selective A1AR antagonist, through the microdialysis probe causes a transient increase in extracellular glutamate levels when infused during the subjective day. These results are all consistent with our working hypothesis. However, we also observed that NOx levels fall to the limit of detection during DPCPX infusion. Obviously, these surprising results demand further experimentation to reconcile. We are currently examining the effects of DPCPX infusion on the extracellular levels of glutamate and NOx during the subjective night under funding from another agency. Part of this work has been reported in abstract form (Cuba et al., 2003).

Adenosine and behavioral state regulation: During microdialysis experiments, the subject animal is housed in a light tight box under controlled lighting conditions and behavior is monitored using an infrared sensitive video camera and recorded to VHS tape. In the current protocol, DPCPX infusion begins at 2 hours prior to lights-out, a time when most animals are sleeping. Infusion of 10 μM DPCPX (lowest concentration tested) through the microdialysis probe into the SCN region consistently produces wakefulness in previously sleeping animals, and in some cases, substantial locomotor activity. Accumulation of adenosine, and activation of A1ARs in the basal forebrain of cats has been proposed to contribute to the homeostatic drive for sleep. These exciting new data suggest that blockade of A1ARs in the SCN region promotes wakefulness and increases locomotor activity in hamsters. We intend to follow up these experiments by recording sleep EEG and locomotor activity in animals before, during and after adenosine and DPCPX infusion via microdialysis.

Compartmentation of signaling cascades in the SCN: Our model proposes that light-induced FOS expression occurs in a population of SCN cells (entrainment cells) that are distinct from the oscillatory population that comprises the circadian clock. As an initial test of this hypothesis, we mapped the distribution of cells in the SCN that express FOS and mPER1 in response to nocturnal light exposure. These experiments were conducted in the mPER1-GFP reporter mouse, which permitted the identification of cells that co-express both markers. The results show that distinct populations of SCN cells express either FOS or mPER1-GFP after a 4 hour light pulse beginning at CT18. Less than 4% of immunostained cells expressed both antigens. Similarly, we have examined the distribution SCN cells that express FOS and p-ERK immunoreactivity after nocturnal light exposure in mice and hamsters. In preliminary experiments, we observed distinct patterns of expression with very little overlap, indicating that these signaling cascades are also activated by light exposure in different cell populations. The next step is to

determine whether light induced ERK phosphorylation is dependent upon NO production. Some of these results have been reported in abstract form (Barengo et al., 2003).

Brain slice neurophysiology: In order to facilitate the investigation of light-induced signaling events in the rodent SCN, we have refined our SCN brain slice preparation to accommodate pharmacological studies of signal transduction in the isolated SCN. Optic nerve stimulation (ONS) increased ERK phosphorylation in the SCN brain slice as determined by western analysis of micropunched SCNs isolated from the stimulated brain slices. Furthermore, ERK phosphorylation in response to either glutamate infusion, or ONS was completely blocked by pretreatment with the MEK inhibitor, U-0126. We are currently examining the effects of adenosine and nitric oxide drugs on ONS-driven signaling in the SCN by quantitative ELISA for p-ERK. The unrestricted access of pharmacological agents to the retinorecipient cells in the brain slice will greatly facilitate progress toward an elucidation of RHT-dependent signaling processes in the isolated SCN. Preliminary data obtained during these experiments has been reported in abstract form (Sigworth et al., 2003).

g. Significance

The work conducted under AFOSR funding under F49620-00-1-0058 has resulted in several significant findings, most of which have been published. Perhaps the most significant unpublished observation is that blockade of A1AR in the SCN region promotes wakefulness. This observation could have important implications for sleep regulation in humans. Another result of notable significance is the demonstration that light exposure activates different signaling cascades in distinct cell populations within the SCN. This observation forms the basis for a more detailed characterization of the cellular organization of the hypothalamic circadian clock.

A detailed understanding of the neurochemical regulation of circadian timing will have significant implications for the diagnosis and treatment of disorders of biological timing and sleep in humans. Ultimately, this knowledge will serve as a foundation for the intelligent design of pharmaceuticals for the treatment of sleep disorders, seasonal affective disorders, jet lag and shift maladaptation syndrome.

5. Personnel Supported

Faculty:	Michael A. Rea, PhD	Principal Investigator	(1999 – 2002)
	E. Todd Weber, PhD	Co-Investigator	(1999 – 2001)
	Richard J. Hallworth, PhD	Consultant	(1999)
	Laura Sigworth, PhD	Postdoctoral Fellow	(2001 – 2002)
Technical Staff:	Matthew J. Cato, MS	Senior Technician	(1999 – 2000)
	Keith Kelleher, MS	Lab Technician	(2000 – 2002)

Students:	Holly Babik	Graduate Student	(2000 – 2002)
	Secil Edozie	Undergraduate	(2000 – 2001)
	Diana Caicedo	Undergraduate	(2000 – 2001)
	Pasha Levin	Undergraduate	(2001 – 2002)
	Cat Vinh Nguyen	Undergraduate	(2001 – 2002)

6. Publications & Abstracts

Rea MA, Pickard GE, (2000) Serotonergic modulation of photic entrainment in the Syrian hamster. Biological Rhythm Research 31: 284 - 314.

Rea MA, Pickard GE, (2000) 5-HT1B receptor agonist inhibits light-induced suppression of pineal melatonin production. Brain Research 858: 424-428.

Rea MA, Elliott KJ, Weber ET, Cato MJ, Hallworth RJ. (2000) On the role of adenosine in the hamster SCN. Soc Res Biol Rhythms Absts.Society for Research on Biological Rhythms, May, 2000.

Elliott KJ, Weber ET, Rea MA. (2001) Adenosine1 receptor agonists inhibit photic phase shifts of the circadian activity rhythm in hamsters. European Journal of Pharmacology 414:45-53.

Rea MA, Kelleher KJ, Colbert CM (2001) Serotonergic Regulation of Retinohypothalamic Neurotransmission in the Hamster Suprachiasmatic Nucleus. Soc Neurosci Abst 27.

Sollars PJ, Ogilvie MA, Rea MA, Pickard GE (2001) The period lengthening effect of constant light on the circadian activity rhythm is amplified in 5HT1B receptor knockout mice. Soc Neurosci Abst 27.

Weber ET, Byrnes GT, Rea MA (2001) A1 Adenosine Receptor Agonists Inhibit Light-Induced Phase Delays in Mice. Soc Neurosci Abst 27.

Hallworth RJ, Cato MJ, Colbert CM, Rea MA. (2002) Presynaptic adenosine A1-like receptors regulate retinohypothalamic neurotransmission in the hamster suprachiasmatic nucleus. J. Neurobiol 52: 230-240.

Sollars PJ, Ogilvie MJ, Rea MA, Pickard GE. (2002) 5-HT1B receptor knockout mice exhibit an enhanced response to constant light. J Biol Rhythms 17: 428-437.

Babik HL, Dryer L, Rea MA (2002) Cloning and characterization of hamster serotonin receptors. Soc Neurosci Abst 28.

Lyons LC, Levenson J, Kjabour O, Rea MA, Eskin A. (2002) Temporal variations in glutamate transporters in the rat brain. Soc Neurosci Abst 28.

Sigworth LS, Rea MA. (2003) Adenosine A1 receptors regulate the response of the mouse circadian clock to light. Brain Res. 960:246-251.

Rea MA, Elliott KJ. Selective serotonin uptake inhibitors block light-induced phase shifts in the hamster. Brain Res (submitted).

Weber ET, Rea MA. Pindolol inhibits light-induced phase shifts through a serotonergic mechanism. J Biol Rhythms (submitted).

Rea MA, Kelleher K, Colbert CS. Presynaptic regulation of retinohypothalamic tract neurotransmission by 5HT7 receptor agonists. J. Neurophys (in preparation)

7. Interactions / Transitions

a. Participation / Presentations:

Rea MA
Feb 2000 "Resetting the Biological Clock"
Career Day speaker, University of Houston, Houston, TX

Rea MA
Apr 2000 "Serotonergic Regulation of Circadian Phase"
Texas Center for Neuropharmacology Symposium, University of Houston, Houston, TX

Rea MA
May, 2000 "On the role of adenosine in the hamster SCN"
Society for Research on Biological Rhythms, Amelia Island, FL

Rea MA
Nov 2000 "Adenosine as a Modulator of Circadian Phase"
College of Optometry, University of Houston, Houston, TX.
Rea MA
Mar 2001 "Serotonergic Regulation of Circadian Timing in Hamsters"
Department of Neuroscience, Baylor College of Medicine

Weber ET
Nov 2001 "A1 Adenosine Receptor Agonists Inhibit Light-Induced Phase" Delays in Mice"
Society for Neuroscience Meeting, San Diego, CA

Rea MA
Nov 2001 "Serotonergic Regulation of Retinohypothalamic Neurotransmission in the Hamster Suprachiasmatic Nucleus"
Society for Neuroscience Meeting, San Diego, CA

Rea MA
Dec 2001 "It's About Time: The Neurobiology of Circadian Timing".
Distinguished Lecturer Series, Creighton University School of Medicine.

Rea MA
Dec 2001 "Presynaptic 5HT7 Receptors on Retinohypothalamic Terminals?"
SECTS for Clocks Symposium, University of Houston.

Rea MA
Mar 2002 "Serotonergic Regulation of Circadian Timing in Hamsters"
College of Pharmacy, University of Houston

Rea MA
May 2002 "Serotonergic Regulation of Circadian Timing in Hamsters"
Keck Eye Institute, University of Southern California

Babik HL
Nov 2002 "Cloning and characterization of hamster serotonin receptors"
Society for Neuroscience,

Rea MA
Dec 2002 "Neurochemical Regulation of Circadian Rhythms in Hamsters"
Creighton University School of Medicine.

b. Consultative and Advisory Functions:

Rea MA

1. Scientific Advisory Board
   AFOSR Program on Human Chronobiology (Harvard University)
   2002

2. Grant Reviewer: AFOSR (2000 – 2001)
   NIMH (2000 – 2003)
   Welcome Trust (2000 – 2001)
   NSF (2001)
   University of Houston (1999 – 2003)
   Texas Higher Education Coordinating Board (2000 – 2003)

3. Consulting: Visigen Biotechnologies, Houston TX

c. Transitions: None

8. New Discoveries, Inventions, Patent Disclosures: None

9. Honors / Awards:

Rea MA
Distinguished Lecturer, Creighton University Medical School
Dec 2001

Figure Legends

Figure 1. Proposed secondary structure of the Syrian hamster 5-HT1A receptor. Amino acids are denoted as single letters according to convention. Yellow indicates amino acids that contribute to transmembrane domains. Blue indicates amino acid differences relative to the published rat sequence.

Figure 2. Proposed secondary structure of the Syrian hamster 5-HT7 receptor. Amino acids are denoted as single letters according to convention. Yellow indicates amino acids that contribute to transmembrane domains. Blue indicates amino acid differences relative to the published rat sequence.

Figure 3. Expression of serotonin receptors in the Syrian hamster SCN. A. Ethidium bromide stained bands of RT-PCR amplification products obtained from hamster SCN mRNA extracts collected at zeitgeber time (ZT) 3, 9, 15, and 21. All four serotonin receptor subtypes were detected in the hamster SCN. B. mRNA for the 5-HT7 receptor shows a pronounced diurnal fluctuation with peak values occurring during the dark phase of the LD 14:10 cycle.

Figure 4. Expression of the 5-HT7 receptor in the Syrian hamster SCN. A. Northern blot showing detection of 5-HT7 receptor mRNA in extracts of the rat (R) and hamster (H) SCN using an antisense, but not sense, probe based upon the published nucleotide sequence of the rat transcript. B. In situ hybridization of antisense (right) and sense (left) riboprobes for the 5-HT7 receptor in coronal sections of the Syrian hamster hypothalamus at the level of the SCN (red arrows). III = third ventricle; OC = optic chiasm.

Figure 5. Pseudocolor images of the binding of [3H] 8-OH-DPAT in the Syrian hamster SCN. Red denotes high density of bound radioligand, while blue represents background (non-specific) binding. Examples of specific binding (top left), non-specific binding (top right), and binding in the presence of a saturating concentration of pindolol (lower left) or ritanserin (lower right), indicating the presence of 5HT1A and 5HT7 receptor sites. Approximately 50% of the binding in the SCN is blocked by either antagonist.

Figure 6. (+/-) 8-OH-DPAT dose-dependently attenuates optic nerve stimulation evoked excitatory post-synaptic currents (EPSCs). A. Inward currents evoked by electrical stimulation in the absence (1) and presence (2) of 5 μM 8-OH-DPAT. The EPSC after 15 minute washout of the drug is also shown (3). B. Graph of EPSC amplitude during application and washout of 8-OH-DPAT (indicated by bar). Data represent the mean +/- S.E.M. of 5 determinations. C. Dose-response curve for 8-OH-DPAT attenuation of EPSC amplitude.

Figure 7. Effect of (+/-) 8-OH-DPAT is blocked by 5-HT7 receptor antagonists. A. Representative EPSCs showing effect of the 5-HT7 receptor antagonist methiothepin on 8-OH-DPAT inhibition of EPSC amplitude. (1) Control, (2) methiothepin, (3) methiothepin + 8-OH-DPAT. B. Graph of EPSC amplitude during application and

washout of methiothepin before and during 8-OH-DPAT (indicated by bars). C. Effect of 5 μM serotonin antagonists on 8-OH-DPAT attenuation of EPSC amplitude. Data represent the mean +/- S.E.M. of 5 determinations. Asterisks denote statistically significant differences relative to 8-OH-DPAT without antagonist.

Figure 8. (+/-) 8-OH-DPAT fails to attenuate glutamate-induced currents in retinorecipient SCN neurons. A. EPSCs evoked in the same neuron in response to optic nerve stimulation (top traces) and glutamate application (bottom traces) before (baseline) and during 8-OH-DPAT application, and after addition of the glutamate receptor antagonist, DNQX. B. Effect of 8-OH-DPAT on current amplitude of EPSCs and glutamate responses. Data represent the mean +/- S.E.M. of 5 determinations. Asterisks denote statistically significant differences.

Table 1: Coding and amino acid sequences of the Syrian hamster 5-HT7C receptor

Consensus h5-HT7 nucleotide sequence

[redacted]ATGGGCGTTAACAGCAGCGGCCGTCCCGACCTCTACGGCCACCTCCGCTCGCTCATCCTGCCGGAGGT
GGGGCACGGGCTGCAAGATCTGAGCCCCGACGGTGGCGCCCACTCGGTGGTGAGCTCCTGGATGCCGCACC
TGCTGAGCGGCGTCCCGGAGGTGACGGCTAGCCCCGCGCCCACCTGGGACGCGCCCCCGGACAATGTCTCC
GGCTGCGGGGAGCAGATCAACTACGGCAGAGTCGAGAAAGTTGTGATCGGCTCCATCCTGACGCTCATCAC
GCTGCTGACGATCGCAGGCAACTGCCTGGTGGTGATCTCGGTGTGCTTCGTCAAGAAGCTCCGCCAGCCCT
CCAACTACTTGATTGTGTCCCTGGCGCTGGCTGACCTCTCGGTGGCCGTGGCGGTCATGCCTTTCGTTAGC
GTCACAGACCTCATCGGGGGCAAGTGGATCTTTGGCCACTTCTTCTGCAACGTTTTCATCGCCATGGACGT
CATGTGCTGCACGGCCTCGATCATGACCCTGTGCGTGATCAGCATCGACAGGTACCTTGGGATCACGAGAC
CCCTCACATACCCTGTGAGGCAGAATGGGAGGTGCATGGCCAAAATGATTCTGTCGGTCTGGCTTCTCTCG
GCCTCCATCACCTTACCTCCGCTCTTCGGATGGGCTCAGAATGTAAACGATGACAAAGTGTGCTTGATCAG
CCAGGATTTTGGCTACACGATCTACTCCACCGCCGTGGCGTTTTATATCCCCATGTCGGTCATGCTGTTCA
TGTACTATCAGATTTACAAGGCCGCCAGGAAGAGCGCGGCCAAACACAAGTTCCCAGGCTTCCCGCGCGTG
CAGCCGGAGAGCGTCATCTCTCTGAACGGCGTGGTGAAGCTCCAGAAGGAGGTGGAGGAATGCGCAAACCT
TTCGAGACTGCTCAAACACGAAAGGAAAAACATCTCCATCTTCAAGAGGGAACAGAAAGCAGCCACCACGT
TGGGGATCATCGTGGGAGCCTTCACAGTGTGCTGGCTGCCGTTTTTCCTCTTGTCCACAGCAAGACCCTTT
ATCTGTGGCACGGCCTGCAGCTGCATCCCGCTGTGGGTGGAGAGGACATGTCTGTGGCTGGGCTATGCAAA
CTCTCTCATTAACCCTTTTATATATGCCTTCTTCAACCGGGACCTGAGGACCACCTACCGCAGCCTACTCC
AGTGCCAGTACAGGAATATCAATCGGAAGCTCTCTGCAGCAGGCATGCACGAGGCCCTGAAACTTGCTGAG
AGGCCTGAGAGAGTCGAGTTTGTGCTCATAACCAGAGCCTCAGGAGTCCAGCAGGCACTCGAGAATTTTCC
TTGGGGTAACGGAGTGAATACAGGGAAAAAGGCTGTGAATACTGTTGCACTGACAAAACTC[redacted]CCACTGT
AGCAAAAAAGGTCATGATTCATGATCG

Consensus h5-HT7 amino acid sequence (similar to rat 5-HT7c)

MM[redacted]VNSSGRPDLYGHLRSLILPEVG[redacted]GLQDLSPDGGAHSVVSSWMPHLLS
mm[redacted]vnssgrpdlyghlrslilpevg[redacted]glqdlspdggahpvvsswmphlls

G[redacted]EVTASPAPTWDAPPDNVSGCGEQINYGRVEKVVIGSILTLITLLTIA
g[redacted]evtaspaptwdappdnvsgcgeqinygrvekvvigsiltlitlltia

GNCLVVISVCFVKKLRQPSNYLIVSLALADLSVAVAVMPFVSVTDLIGGK
gnclvvisvcfvkklrqpsnylivslaladlsvavavmpfvsvtdliggk

WIFGHFFCNVFIAMDVMCCTASIMTLCVISIDRYLGITRPLTYPVRQNG[redacted]
wifghffcnvfiamdvmcctasimtlcvisidrylgitrpltypvrqng[redacted]

CMAKMILSVWLLSASITLPPLFGWAQNVNDDKVCLISQDFGYTIYSTAVA
cmakmilsvwllsasitlpplfgwaqnvnddkvclisqdfgytiystava

FYIPMSVMLFMYYQIYKAARKSAAKHKFPGFPRVQPESVISLNGVVKLQK
fyipmsvmlfmyyqiykaarksaakhkfpgfprvqpesvislngvvklqk

EVEECANLSRLLKHERKNISIFKREQKAATTLGIIVGAFTVCWLPFFLLS
eveecanlsrllkherknisifkreqkaattlgiivgaftvcwlpfflls

TARPFICGT[redacted]CSCIPLWVERTCLWLGYANSLINPFIYAFFNRDLRTTYRS
tarpficgt[redacted]csciplwvertclwlgyanslinpfiyaffnrdlrttyrs

LLQCQYRNINRKLSAAGMHEALKLAERPER[redacted]EFVL[redacted]TRASGVQQALENFP
llqcqyrninrklsaagmhealklaerper[redacted]efvl[redacted]trasgvqqalenfp

WGNGVNTGKKAVN[redacted]VALTKL*PL*QKRS*FMI
wgngmkagikavn[redacted]valtkl

Table 2: Coding and amino acid sequences of the Syrian hamster 5-HT1A receptor

Consensus h5-HT1A nucleotide sequence

[redacted]GATATGTTCAGTCTTGGCCAGGGCAACAATACCACATCGTCCCAGGAGCCCTTCGGGACAGGCGGCAA
CGTTAGTGGCATCTCCGACGTGACCTTCAGCTACCAAGTGATCACCTCTTTGCTTCTGGGCACGCTCATTT
TCTGCGCGGTGCTCGGTAATGCCTGCGTGGTGGCTGCCATCGCCCTGGAGCGCTCCCTCCAGAATGTGGCC
AACTATCTTATCGGCTCCTTGGCGGTCACCGACCTCATGGTGTCAGTGCTGGTCCTGCCCATGGCTGCACT
GTACCAGGTGCTCAACAAGTGGACTCTGGGCCAGGTCACCTGCGACCTGTTTATCGCCCTGGATGTGTTGT
GCTGCACCTCGTCCATCCTGCACCTGTGCGCCATCGCGCTAGACAGGTACTGGGCAATCACTGACCCTATA
GACTACGTGAACAAGAGGACGCCCCGGCGCGCTGCTGCGCTGATCTCGCCCACTTGGCTCATTGGCTTTCT
CATCTCCATCCCGCCCATGCTGGGCTGGCGCACCCCGGAAGACCGCTCGGACCCCGATGCATGCACCATCA
GCAAGGATCACGGCTACACCATCTACTCCACTTTCGGCGCTTTCTATATCCCGCTGCTGCTCATGCTGGTT
CTCTATGGGCGCATCTTCCGAGCCGCGCGCTTTCGGATCCGCAAGACTGTGAAGAAGGTAGAAAAGAAGGG
AGCAGGCACGCGCCTCAGTACATCTTCGGCCCCGCCCCCCAAGAAGAGCTTGAACGGACAGCCAGGTAATG
GGGACTGGAGGCGCAGCGCTGAGAGCAGGGCTGTGGGGGCTCCGTGCGCAAATGGCGCGGTTAGGCAGGGT
GATGACGATGCCACCTTGGAGGTGATCGAGGTGCACCGAGTGGGTAACTCCAAAGACCACCTGCCGCTGCC
CAGCGAGTCGGGAGCTACCTCCTATGCCCCCACCTGCCTGGAGAGAAAAAATGAGCGGAATGCTGAGGCAA
AGCGCAAGATGGCCCTGGCCCGTGAGAGGAAGACAGTGAAGACGCTGGGCATCATTATGGGCACTTTCATC
CTCTGCTGGCTGCCCTTTTTCATTGTGGCTCTGGTCTTACCTTTCTGTGAGAGCAGCTGCCACATGCCTGC
CTTGTTGGGTGCCATAATCAACTGGCTGGGCTATTCCAACTCTCTGCTCAACCCAGTTATTTATGCTTATT
TCAACAAGGACTTTCAAAGTGCGTTTAAGAAGATCATCAAGTGCAAGTTCTGCCGCCGA[redacted]

Consensus h5-HT1A amino acid sequence

MD[redacted]FS[redacted]GQGNNTT[redacted]SQEPFGTGGNV[redacted]ISDVTFSYQVITSLLLGTLIFCAVLGNACVVAAIALERSLQNVA
md[redacted]fs[redacted]gqgnntt[redacted]sqepfgtggnv[redacted]isdvtfsyqvitslllgtlifcavlgnacvvaaialerslqnva

NYLIGSLAVTDLMVSVLVLPMAALYQVLNKWTLGQVTCDLFIALDVLCCTSSILHLCAIALDRYWAITDPI
nyligslavtdlmvsvlvlpmaalyqvlnkwtlgqvtcdlfialdvlcctssilhlcaialdrywaitdpi

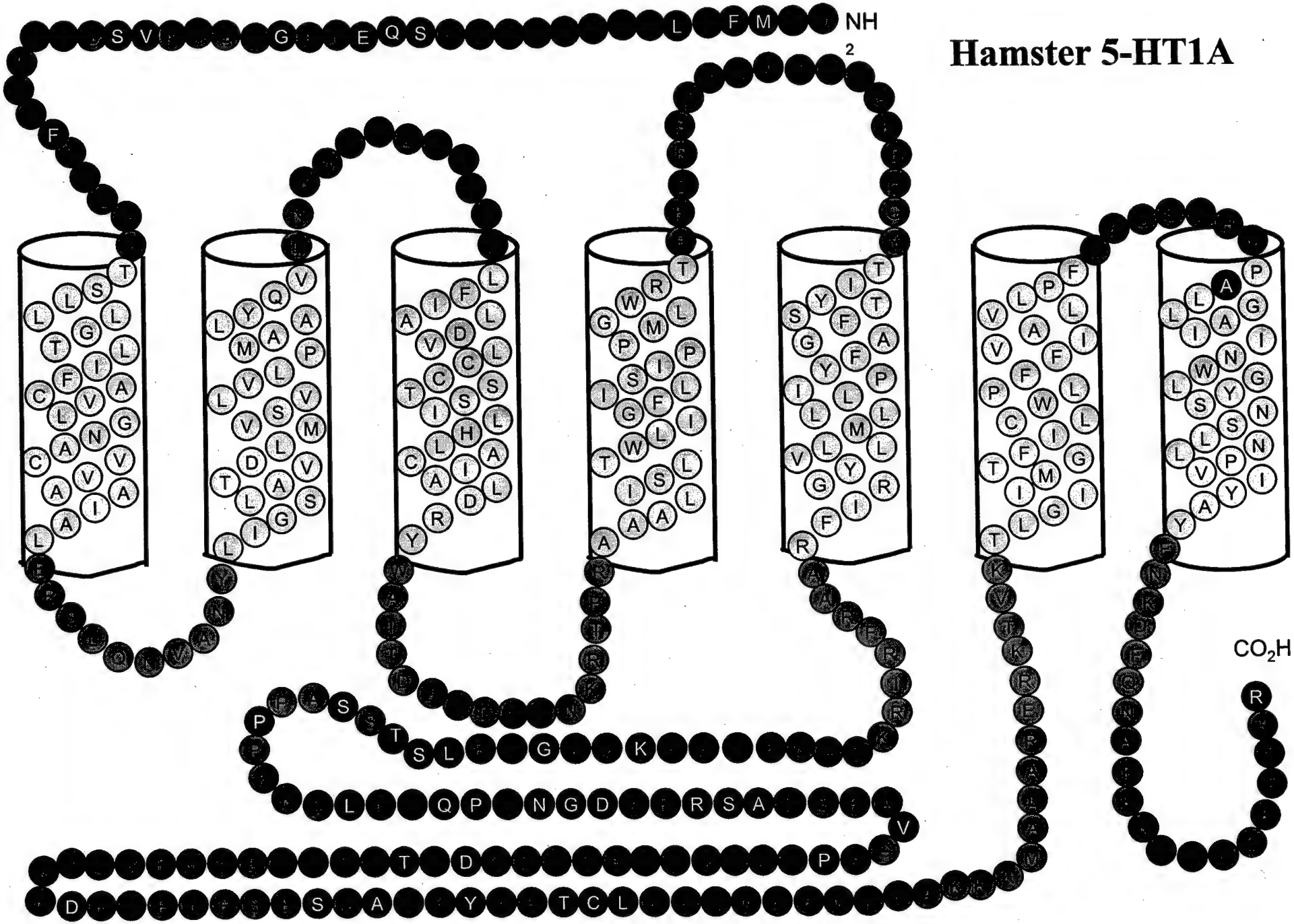
DYVNKRTPRRAAALIS[redacted]TWLIGFLISIPPMLGWRTPEDRSDPDACTISKDHGYTIYSTFGAFYIPLLLMLV
dyvnkrtprraaalis[redacted]twligflisippmlgwrtpedrsdpdactiskdhgytiystfgafyiplllmlv

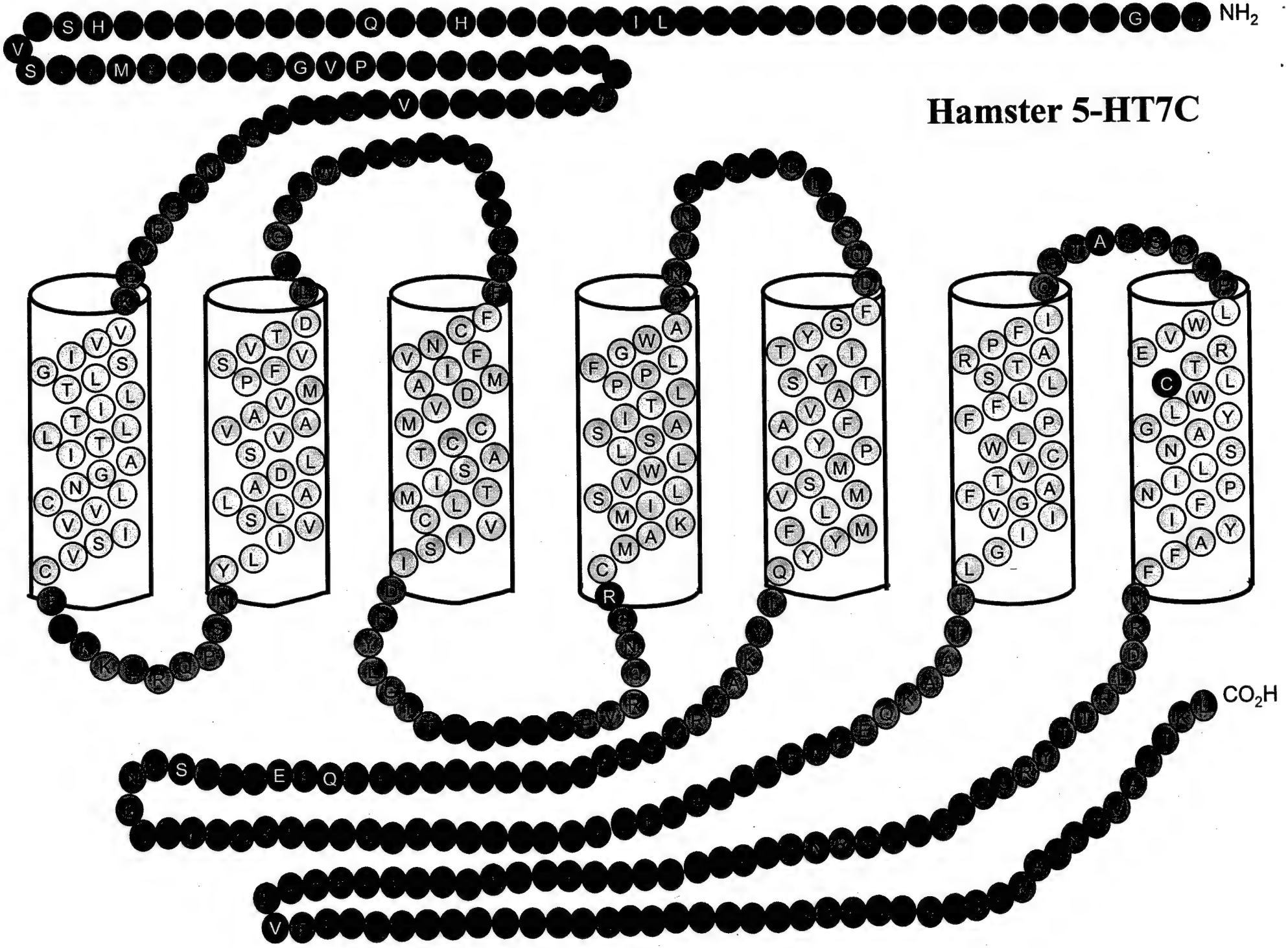
LYGRIFRAARFRIRKTVKKVEKKGAGT[redacted]LSTSSAPPPKKSLNGQPG[redacted]GDWRR[redacted]AE[redacted]RAVG[redacted]PC[redacted]NGAVRQG
lygrifraarfrirktvrkvekkgagt[redacted]lgtssapppkkslngqpg[redacted]gdwrr[redacted]ae[redacted]ravg[redacted]pc[redacted]ngavrqg

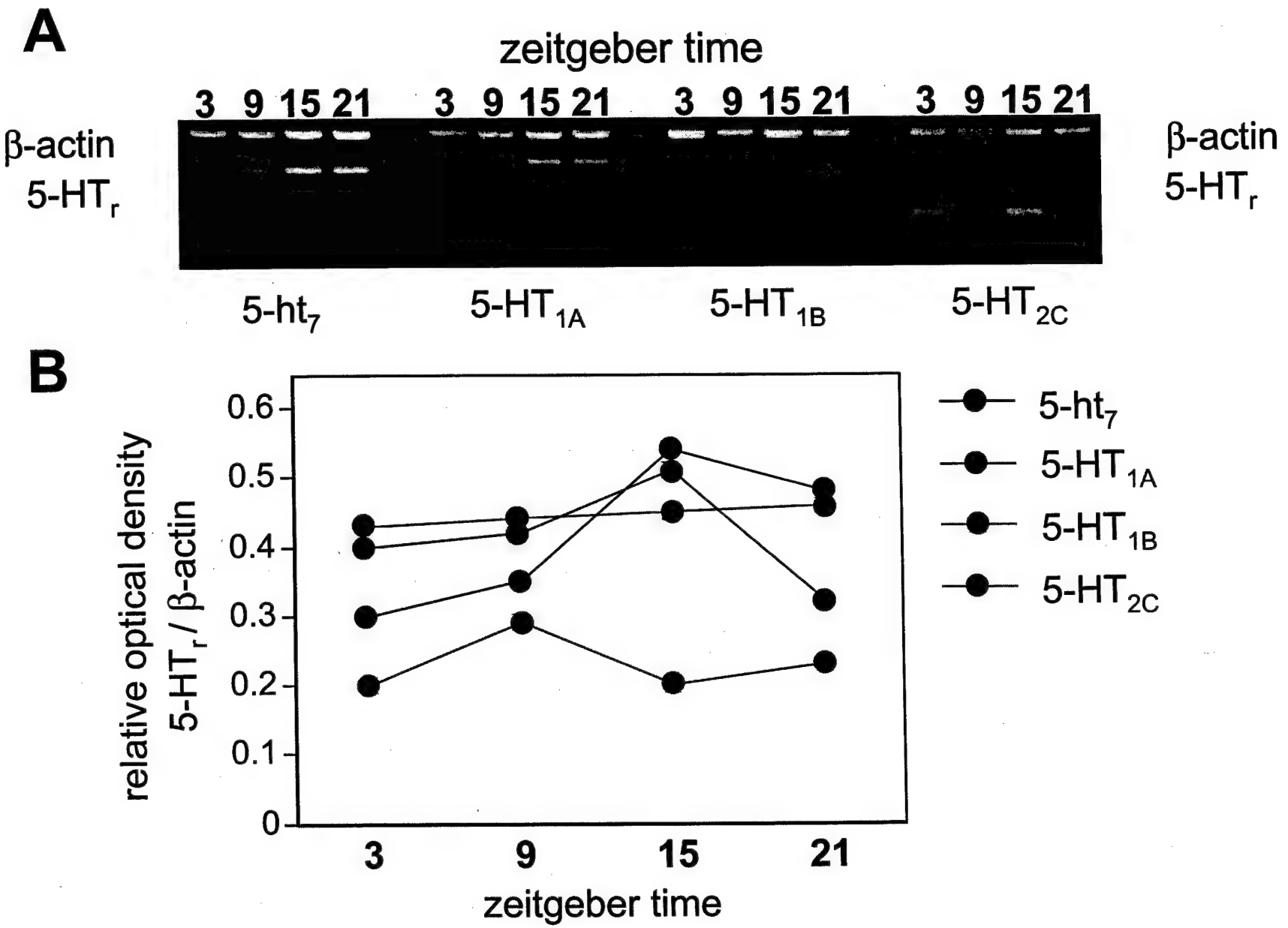
DD[redacted]ATLEVIEVHRVGNSK[redacted]HLPLPSESG[redacted]SYAP[redacted]CLERKNERNAEAKRKMALARERKTVKTLGIIMGTFI
dd[redacted]atlevievhrvgnsk[redacted]hlplpsesg[redacted]syap[redacted]clerknernaeakrkmalarerktvktlgiimgtfi

LCWLPFFIVALVLPFCE[redacted]SCHMPALLGAIINWLGYSNSLLNPVIYAYFNKDFQ[redacted]AFKKIIKCKFCRR
lcwlpffivalvlpfce[redacted]schmpallgaiinwlgysnsllnpviyayfnkdfq[redacted]afkkiikckfcrr

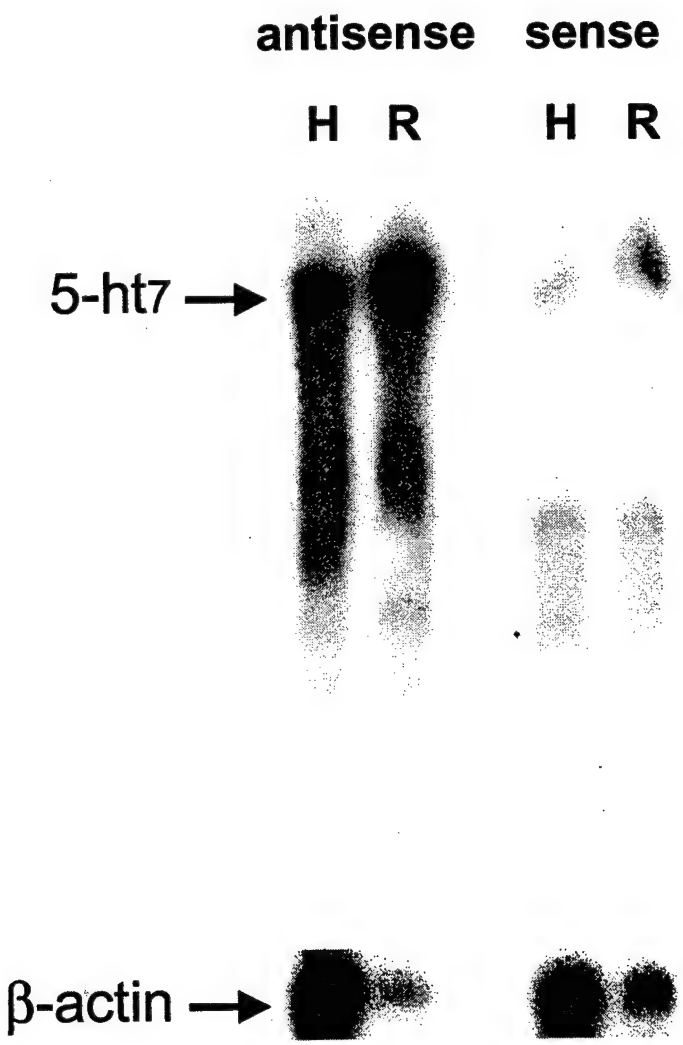
[redacted] and [redacted] highlights indicates the [redacted] and [redacted] codons respectively. [redacted] highlights indicate differences between the hamster (upper case) and rat (lower case) amino acid sequences.


Hamster 5-HT1A
NH2
CO2H

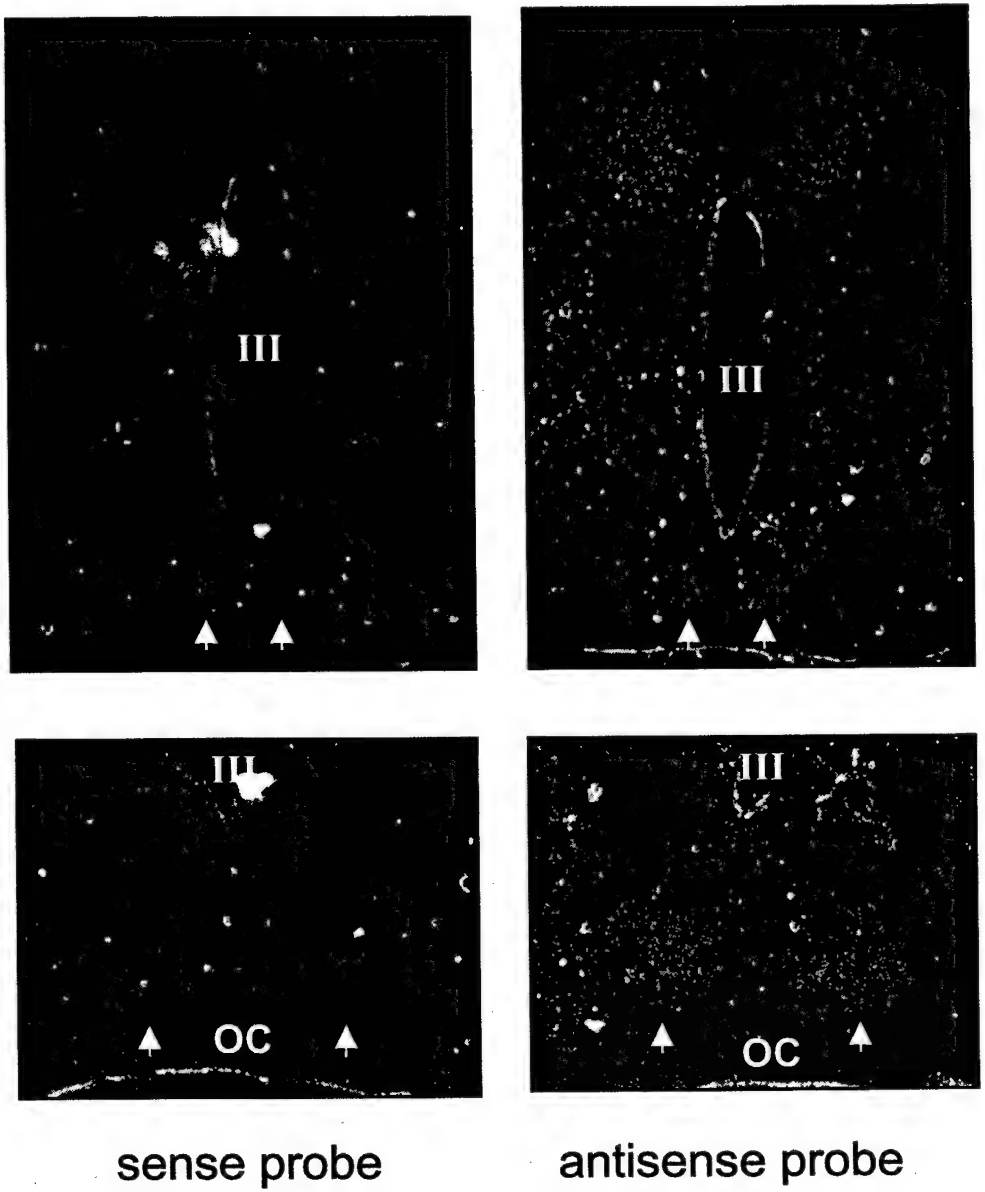

NH2
Hamster 5-HT7C
CO2H


A
zeitgeber time
3 9 15 21 3 9 15 21 3 9 15 21 3 9 15 21
β-actin
5-HTr
β-actin
5-HTr
5-ht7
5-HT1A
5-HT1B
5-HT2C
B
relative optical density
5-HTr / β-actin
0.6
0.5
0.4
0.3
0.2
0.1
0
3
9
15
21
zeitgeber time
5-ht7
5-HT1A
5-HT1B
5-HT2C

A.

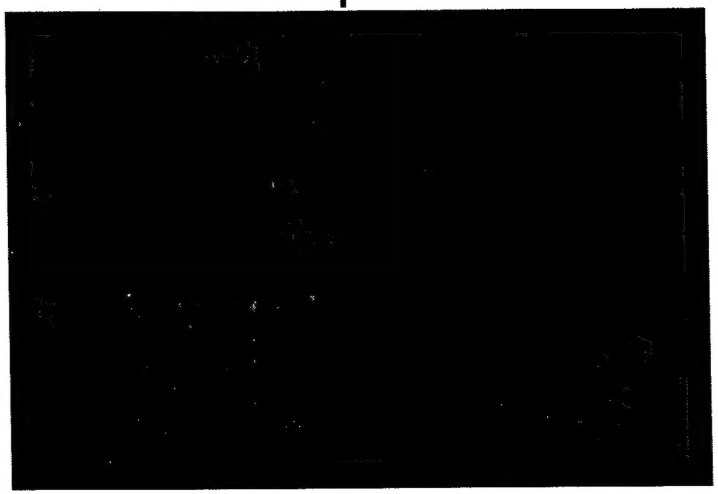

antisense
sense
H R H R
5-ht7
β-actin

B.

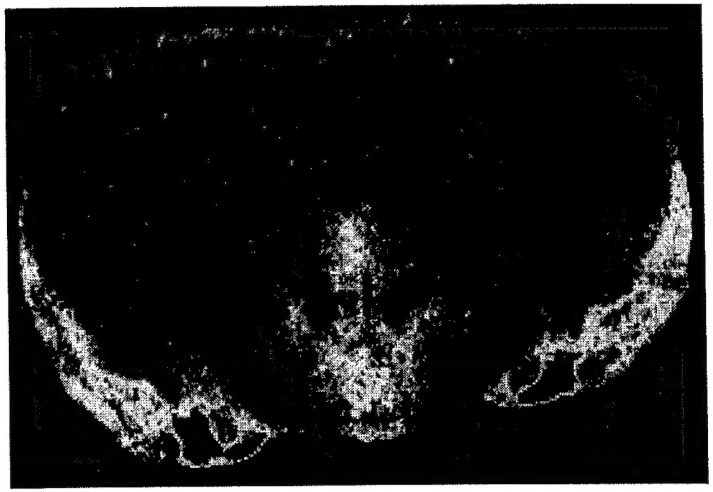

III
III
III
III
OC
OC
sense probe
antisense probe

Total

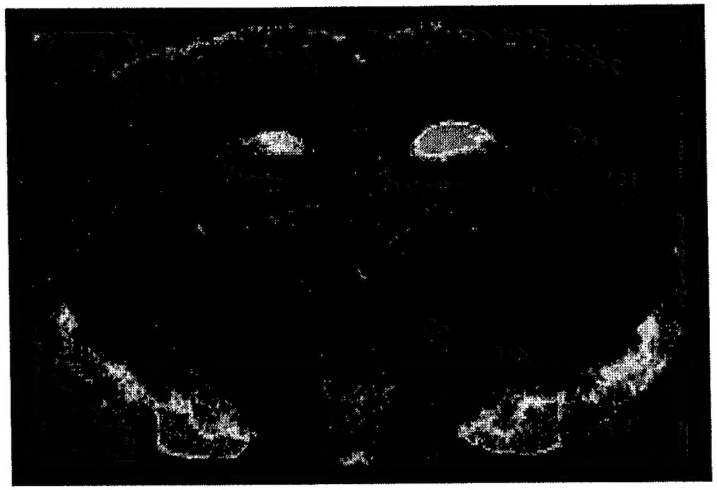
Nonspecific

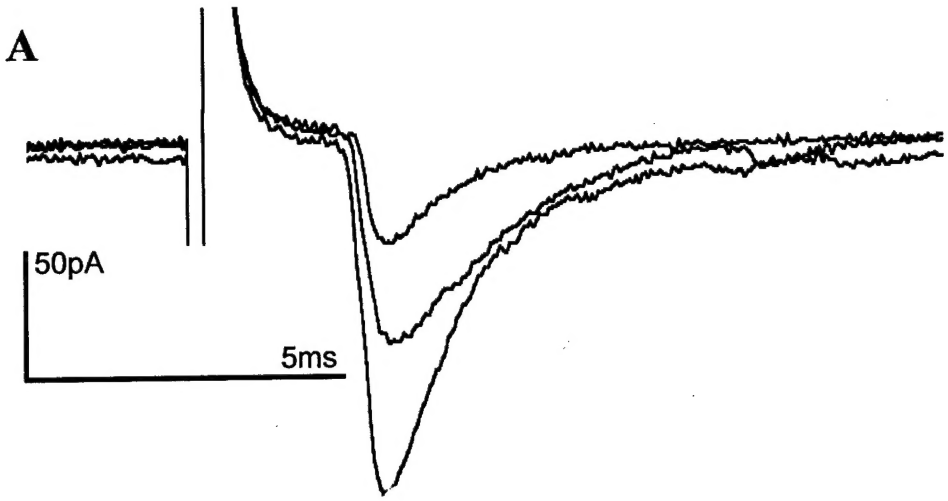


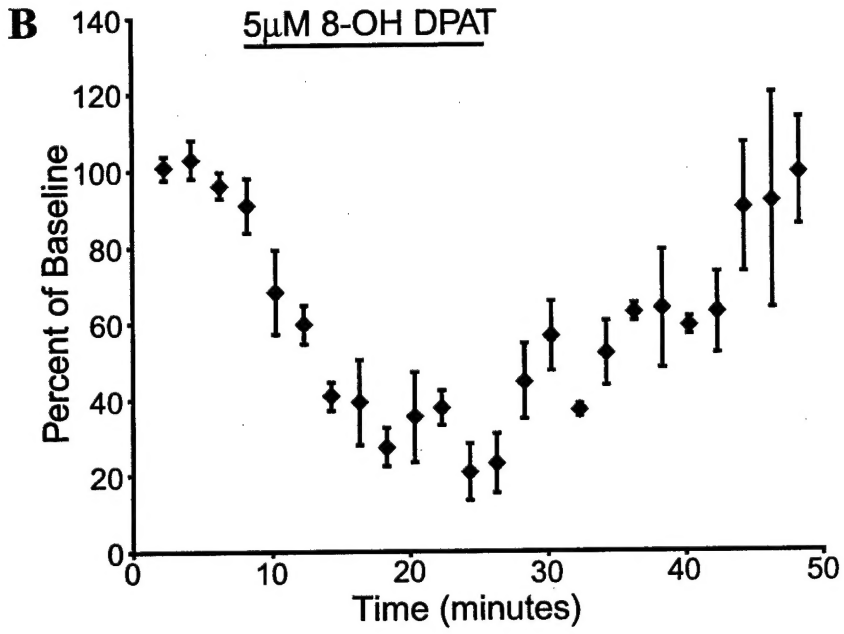
+ Pindolol

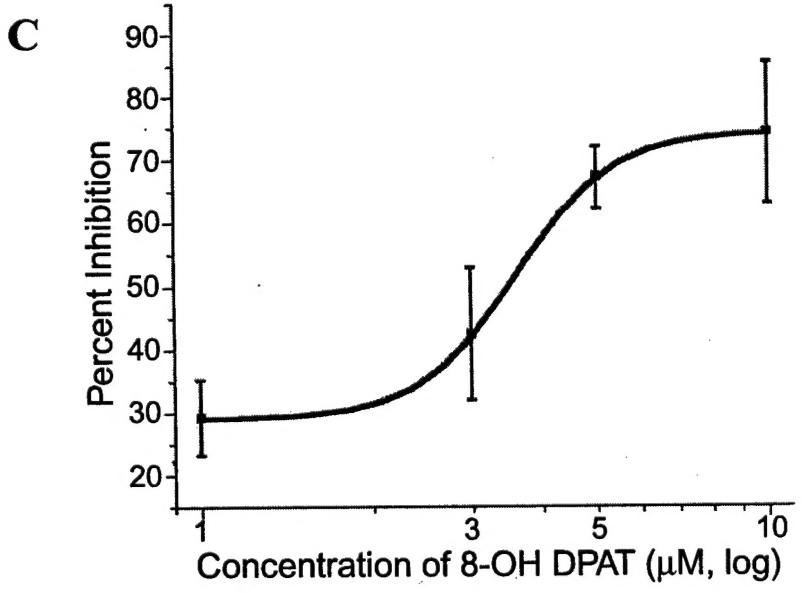


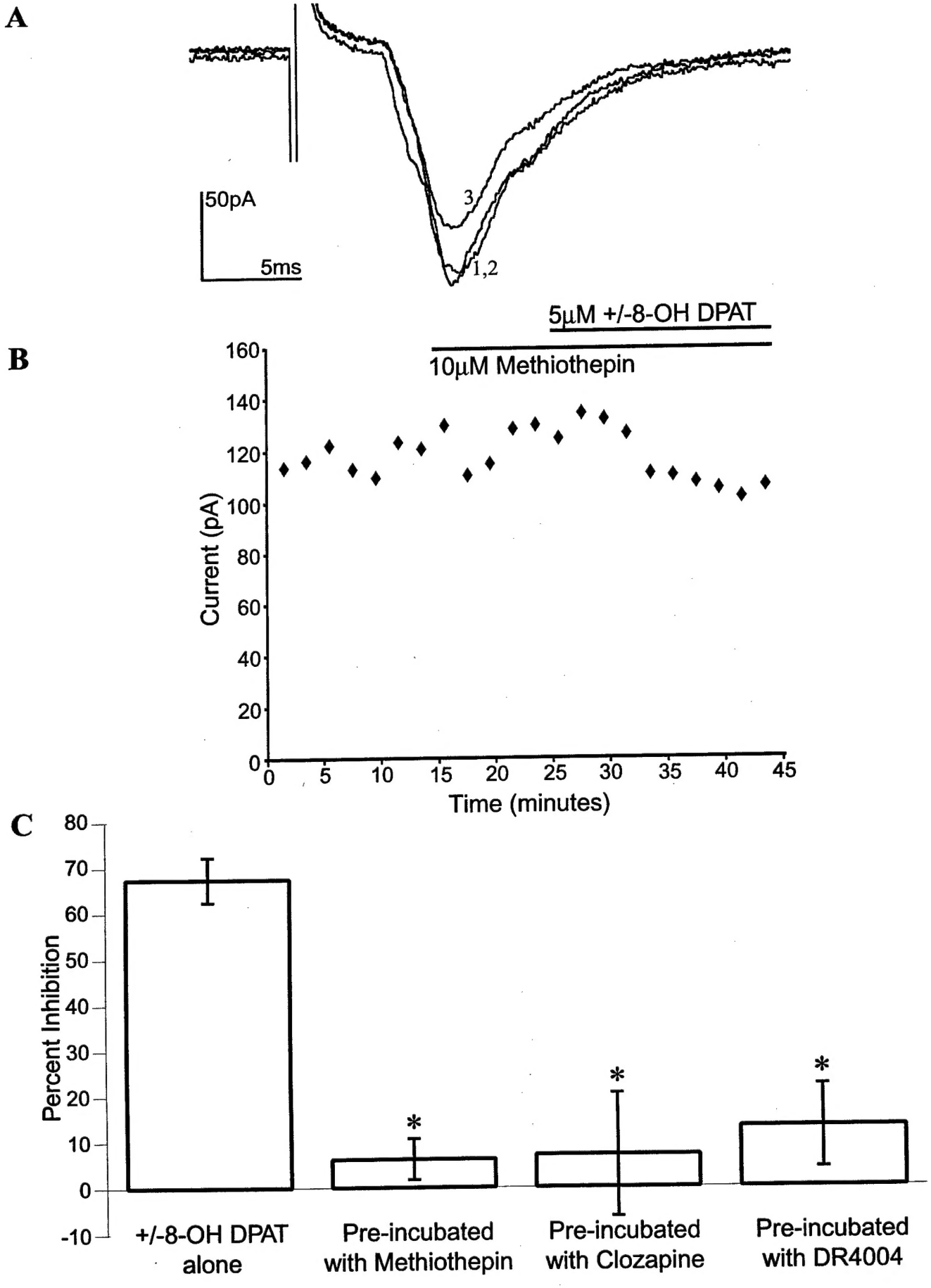
+ Ritanserin

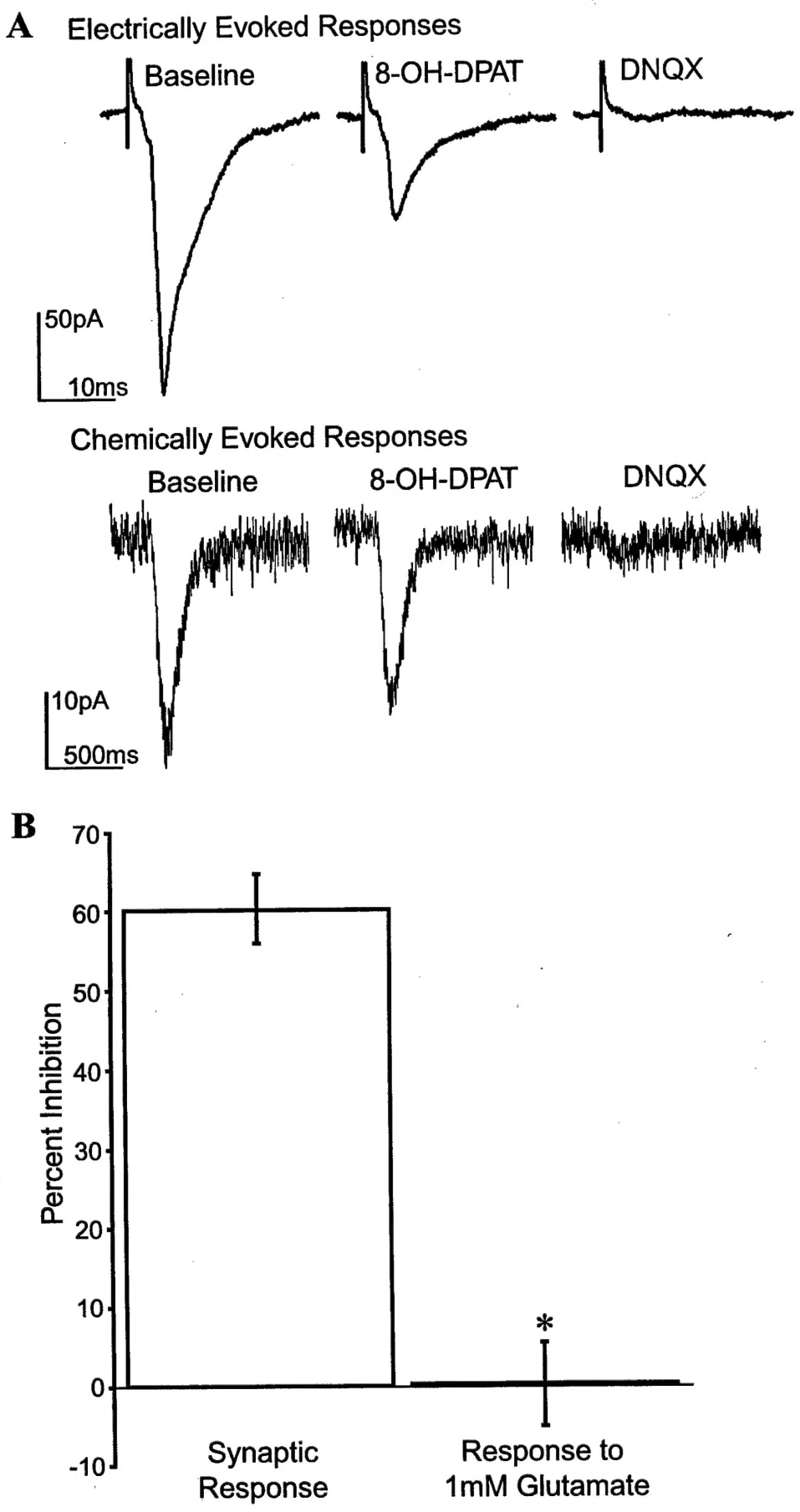



A
50pA
5ms


B
5μM 8-OH DPAT
Percent of Baseline
140
120
100
80
60
40
20
0
0
10
20
30
40
50
Time (minutes)


C
Percent Inhibition
90
80
70
60
50
40
30
20
1
3
5
10
Concentration of 8-OH DPAT (μM, log)


A
50pA
5ms
3
1,2
B
5μM +/-8-OH DPAT
10μM Methiothepin
Current (pA)
Time (minutes)
C
Percent Inhibition
+/-8-OH DPAT alone
Pre-incubated with Methiothepin
Pre-incubated with Clozapine
Pre-incubated with DR4004


A
Electrically Evoked Responses
Baseline
8-OH-DPAT
DNQX
50pA
10ms
Chemically Evoked Responses
Baseline
8-OH-DPAT
DNQX
10pA
500ms
B
Percent Inhibition
70
60
50
40
30
20
10
0
-10
*
Synaptic Response
Response to 1mM Glutamate